Arena Size, Hole Density, and Capture of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) in Grain Probe Traps

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ABSTRACT The relationship of size of test arena, number of holes in a grain probe trap body and capture of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), was determined in simulated field tests conducted in an outdoor screen enclosure exposed to natural temperature fluctuations. Polyvinylchloride (PVC) probe bodies were attached to electronic sensor heads, and insect captures were recorded electronically using an electronic grain probe insect counter (EGPIC) system. In comparisons among PVC probe trap bodies with 60, 132, 252, and 492 holes, tested at 18 insects per kilogram in 4.5, 17, and 40 kg of soft wheat in cylindrical arenas (10.2, 20.3, and 30.5 cm in diameter, respectively), number of holes in the probe trap body had no effect on insect capture, but percentage of insects recovered was indirectly related to size of the test arena. Periodicity of insect capture was determined using the time-stamp data that were recorded by the EGPIC system. Circadian rhythm was observed in the periodicity of the capture that corresponded to foraging activity peaks documented for sawtoothed grain beetles, with activity peaks occurring early in the scotophase. There were shifts in times of peak activity among the different test arena sizes that corresponded to differences in temperature in the grain mass. Increases in both temperature and contact between insects and grain probe in the smallest arenas resulted in higher capture of sawtoothed grain beetles. This research documents additional important factors when evaluating capture of sawtoothed grain beetles in grain probe traps.

KEY WORDS *Oryzaephilus surinamensis*, automated monitoring, grain probe trap, bioassay conditions, diel rhythm

GRAIN PROBE TRAPS WERE developed from pitfall traps for sampling insect pests in stored grain. Pitfall traps capture soil surface-dwelling insects, which are intercepted as they move along the ground and fall into the trap. Some of the first traps used in stored grain were simple pitfall traps that were placed at the grain surface to capture insects moving across the grain (Watters and Cox 1957). Pitfall traps work well to monitor insects that are concentrated at the grain surface but do not permit sampling insects in the grain mass. To trap insects deeper in the grain mass, Loschiavo and Atkinson (1967) developed a modified pitfall trap that included an elongated cylinder with holes drilled into the sides that were above a funnel and insect receptacle. The cylinder is pushed into the grain mass and insects moving through the grain enter the cylinder and are retained in the receptacle. Numerous improvements have been made to the initial trap designs for both pitfall traps and probe traps, and a number of these traps are commercially available (Cogan et al. 1990, White et al. 1990).

Difficulties in deploying traps in grain bins and the necessity of frequently reentering the grain bin to collect insects and to service the traps have impeded using traps for management decisions. The electronic grain probe insect counter (EGPIC; Shuman et al. 1996, Litzkow et al. 1997) is an automated monitoring system developed to overcome these limitations. EGPIC uses grain probe traps that are modified by the addition of infrared-beam sensor heads attached to the bottom of the probe trap bodies. An electronic count is generated whenever an insect that has crawled into a probe trap falls through the sensor head, and this information is transmitted to a computer via beam generation/detection circuitry. Field tests of prototype EGPIC systems have been promising (Arbogast et al. 2000, Shuman et al. 2001, Toews et al. 2003); however, the probes used in these systems were too cost-prohibitive for commercial use. The prototype version has a precision-milled sensor head (Epsky and Shuman 2000) and a commercially available probe trap (grain probe insect trap, ThermoTrilogy Corp., Columbia, MD) used in the inverted position (Subramanyam et al. 1989) for the probe trap body. To be

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more cost-effective, the sensor head and the probe trap body could be manufactured as a single multi-piece injection-molded unit. As part of research on design changes for an injection-molded trap body, studies were conducted to evaluate the relationship between number of holes on the probe trap body and insect capture.

A number of factors have been found to affect insect capture in probe traps, including insect density, trap depth, attractants (White and Loschiavo 1986), insect species, trapping duration, grain temperature, grain type and condition, and trap placement (Cuperus et al. 1990). Previously, we found a direct relationship between number of holes (from 60 to 492 holes along a 40-cm-long trapping surface) and capture of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and the rice weevil, *Sitophilus oryzae* (L.), but no effect on capture of the red flour beetle, *Tribolium castaneum* (Herbst) (Epsky and Shuman 2002). Toews and Phillips (2002) found that increase in number of holes (from 40 to 120 holes along a 15-cm-long probe body) did not affect capture of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). Among other factors, one of the differences between the two studies was the size of the test arena. Our studies were conducted in 10-cm-diameter cylinders containing wheat (2.8 kg) and the study by Toews and Phillips (2002) used 28-cm-diameter buckets containing wheat (17 kg). A wide range of arenas have been used for evaluation of probe traps, ranging from Plexiglas cylinders that were 9.7 cm in diameter by 43 cm (containing 1.9 kg of wheat; Shuman et al. 1996) to grain elevators (White et al. 1990). Little is know about the relationship between arena size and probe trap efficacy, and how arena size may affect comparisons among probe traps. Therefore, studies were conducted to further study the effect of change in number of holes in the probe trap body and insect capture, but using arenas that varied from 10 to 30 cm in diameter. These studies were conducted using sawtoothed grain beetles, the insect species most dependent on initial conditions in previous research (Epsky and Shuman 2002).

Materials and Methods

Insects used in this study were 2–4-wk-old adult sawtoothed grain beetles. Insects were obtained from a laboratory colony that has been maintained at the USDA–ARS laboratory in Gainesville, FL, for at least 20 yr. Insects were maintained on a photoperiod of 16:8 (L:D) h with scotophase starting at 1200 hours. Wheat used in this study was organic soft wheat. After a trial, the wheat was sieved (10-mesh screen) to remove all insects, frass, and feeding debris. Grain was kept in a freezer between tests to kill all eggs and developing larvae. Grain removed from the freezer was transferred to shallow trays, which were held at room temperature for 24 h before use.

Probe bodies were produced from dark gray polyvinylchloride (PVC) cylinders (2.5 cm in diameter). The probe bodies were fabricated using a computer-controlled step and repeat drilling apparatus custom designed by Analytical Research Systems, Inc. (Gainesville, FL). The 2.79-mm-diameter holes were precision-drilled at an upward 45° angle on the 40-cm-long trapping surface. The probe bodies had 12 columns and 5, 11, 21, or 41 holes per column for a total of 60, 132, 252, or 492 holes. These probe bodies were threaded to prototype precision-milled sensor heads and insect counts were recorded electronically.

Tests comparing insect capture among different probe bodies were conducted in arenas made from cylinders of varying diameter (i.d.) PVC irrigation pipe (PWPipe, Eugene, OR) with an endcap glued to the base to form an arena. The three cylindrical arena sizes tested were ≈10.2 cm in diameter by 68 cm, ≈20.3 cm in diameter by 74 cm, and 30.5 cm in diameter by 84 cm. These held 4.5, 17, and 40 kg of grain, respectively. A single grain probe trap was placed in the center of the arena with the bottom touching the bottom of the arena, and grain was added to ≈1 cm above the top row of holes. Thus, the depth of the grain sampled was the same in all arenas and a line of liquid Teflon (polytetrafluoroethylene) was added around the inside perimeter of each arena to prevent insects from moving into the area above the grain. A sleeve made from clear acetate sheeting was placed around the probe body to prevent insects from entering the probe traps before the electronic counts were initiated. The trap receptacle was coated with Teflon to prevent captured insects from moving back into the sensor probe. Insects were added to the top surface of the grain and plastic wrap was placed over the top of the arena to prevent test insects from escaping. Insect density was kept constant at 18 insects per kilogram for all arena sizes, for a total of 81, 320, and 723 sawtoothed grain beetles in the 10.2-, 20.3-, and 30.5-cm-diameter arenas, respectively. After 24 h to allow the insects to disperse, sleeves were lifted and electronic counting was initiated. Number of insects captured was determined by emptying the trap receptacle after an additional 72 h. Electronic insect counts were used to compare periodicity of insect capture among the different treatments.

Tests were conducted in a screen enclosure so that arenas were exposed to natural temperature fluctuations. The screen enclosure was attached to a portable building, which housed a computer that recorded the electronic counts. The screen enclosure had an insulated roof so arenas were not exposed to direct sunlight and the arenas were placed close to the exterior wall of the portable building so they were protected from rainfall. Temperature probes were added to each arena, with the sensor placed next to the probe trap at the midpoint of the trapping surface and temperature was recorded every 1.5 min (HOBO data logger, Onset Corp., Bourne, MA) during an experiment. For comparative purposes, a temperature probe was placed in an empty arena placed next to the test arenas. To get three replicates for all combinations of number of holes and arena size treatments, there were 12 wk of tests conducted in four consecutive blocks of

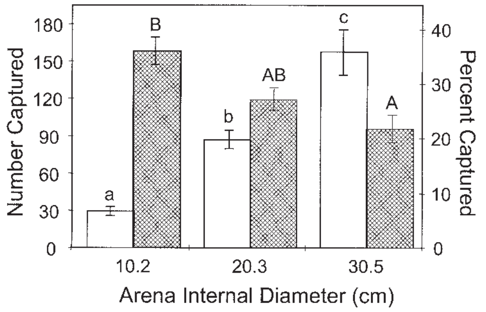

Fig. 1. Mean (±SD) number (open bars) and percentage (hatched bars) of capture of sawtoothed grain beetles in grain probe traps placed in cylindrical arenas that were 10.2-, 20.3-, and 30.5-cm i.d. Insect density was 18 beetles per kilogram in all arenas, there were 4.5, 17, and 40 kg of soft wheat per arena, respectively, and capture was recorded after 72 h of trapping. Means headed by the same case letter are not significantly different [$P = 0.05$, Tukey's HSD mean test on $\log(x + 1)$ transformed data, nontransformed means shown), separate analyses were conducted for number and percentage data.

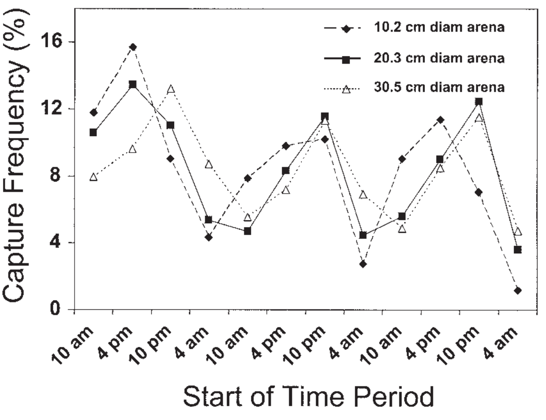

Fig. 2. Periodicity of capture of sawtoothed grain beetles in tests conducted in cylindrical arenas that were 10.2- (solid diamond, dashed line), 20.3- (solid square, solid line) and 30.5 (open triangle, dotted line)-cm i.d. All tests were started at 10 a.m. ± 1 h. A total of 255, 892, and 1642 sawtoothed grain beetles was captured in the tests conducted in the 10.2-, 20.3-, and 30.5-cm diameter arenas, respectively.

3-wk periods. Tests were conducted from June to October 2001.

Numbers of insects captured and percent of insects recovered were analyzed by separate three-way analysis of variance (ANOVA) with all two-factor interactions using block, number of holes and arena diameter as factors (Proc GLM, SAS Institute 1985). Data were subjected to the Box–Cox procedure, which is a power transformation that regresses log-transformed standard deviations (y) against log-transformed means (x) (Box et al. 1978), and data were transformed [$\log(x + 1)$] to stabilize the variance before analysis. Nontransformed means are presented. Mean comparisons were conducted using Tukey's honestly significant difference (HSD) ($P = 0.05$). Information from time-stamped electronic insect counts recorded by the EGPIC system was evaluated by partitioning capture into consecutive 6-h periods and determining capture frequency over the 72 h of a test.

Results

Number of holes in the probe trap and block had no effect on insect capture ($F = 0.27$; df = 3, 12; $P = 0.8483$ and $F = 2.92$; df = 3, 12; $P = 0.0776$, respectively), and there were no interactions between any two factors. Size of the arena, however, did affect insect capture ($F = 47.19$; df = 2, 12; $P < 0.0001$) and number of insects captured increased with the increase in arena size (Fig. 1). Percentage of insects recovered was also affected by size of arena only ($F = 9.82$; df = 2, 12; $P = 0.0030$) and percentage decreased as arena size increased (Fig. 1).

Periodicity of insect capture was determined using the time-stamp data that were recorded by the EGPIC system. Total capture for each species was partitioned into number captured for each 6-h period beginning at 10 a.m. on day 1 (±1 h depending on the individual trial) and ending at 10 a.m. on day 3. In one of the tests, there were overcounts, that is, more electronic counts than number of insects captured by that probe (38 electronic counts, 26 insects captured). For all other tests, there were either more or an equal number of insects captured than electronic counts recorded (probe accuracy ranged from 87 to 100%); thus, data from the single test with the overcount were deleted from subsequent analysis. The periodicity of insect capture in the different sized arenas is shown in Fig. 2. Peaks in capture occurred in the first 12 h after the sleeves were removed in tests in the 10.2- and 20.3-cm-diameter arenas, with a lag in peak capture in the 30.5-cm-diameter arena. Broad peaks in capture were observed late in the day in the 10.2-cm-diameter arena for the next 2 days. For tests in the 20.3- and 30.5-diameter arenas, peak capture was sharper (on days 2 and 3) and tended to occur after the peak capture in the smallest arena (on day 3). One reason for the differences in time of peak capture may be difference in grain temperature among the arenas such as that observed in the test conducted during the week of 25 June 2001 (Fig. 3). Temperature in the grain in the 10.2-cm-diameter arena was similar to the ambient temperature recorded in an empty cylindrical arena. The larger grain masses in the 20.3- and 30.5-cm-diameter arenas took longer to heat up and to cool down, and these lags in temperature change parallel the lags in peak capture (on day 3). Peaks in insect activity tended to correspond with the onset of scotophase that occurred during insect rearing early in the trial. By the end of the trial, activity peaks tended to correspond with the ambient scotophase. Thus, the insects may have acclimated to the subtle differences in photoperiod within a few days.

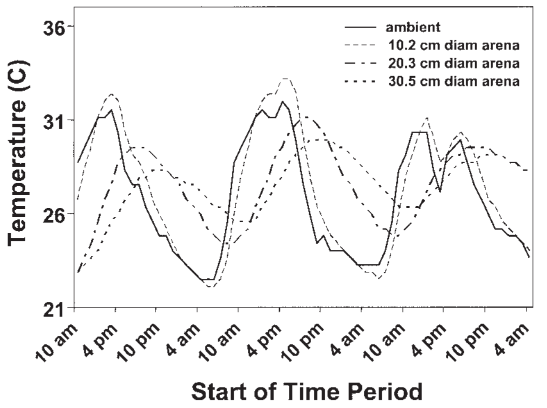

Fig. 3. Temperature recorded on data loggers from temperature probes placed next to probe traps in wheat-filled cylindrical arenas that were 10.2- (dashed line), 20.3- (dotted-dashed line), and 30.5 (dotted line)-cm i.d. to record grain temperature, or placed in an empty arena to record ambient air temperature (solid line).

Discussion

Unlike our previous study (Epsky and Shuman 2002), there was no relationship between number of holes in the probe trap and sawtoothed grain beetle capture even though similar sized arenas were used in both studies. However, the earlier study used 71 insects per kilogram of wheat versus 18 insects per kilogram of wheat in the study reported herein. In the study conducted with *C. ferrugineus,* in which there was no relationship between number of holes and insect capture (Toews and Phillips 2002), an even lower density of two insects per kilogram of wheat was tested. Thus, as has been observed in previous studies, insect density is an important factor in number of insects captured by grain probe traps and thus is an important factor when considering comparative efficacy of different trapping systems for stored grain insect pests. Size of the test arena also affected grain probe trap efficacy. Although total number of insects captured increased, we found that sawtoothed grain beetle capture rate (i.e., percentage of capture) decreased as arena size increased. This has also been indicated in tests with *C. ferrugineus,* because capture rate decreased from 63% in 24 h in tests conducted in 1.9 kg of wheat (Shuman et al. 1996) to 40% in 7 d in tests conducted in ≈6 kg of wheat (estimated from arena dimensions; Chemelli 1974) and 10–14% in 3 d in tests conducted in ≈27 kg of wheat (Toews and Phillips 2002). Fargo et al. (1989) noted that anything that increases insect contact with the trap increases capture. Thus, the increase in arena size in our study resulted in an increase in grain volume, which subsequently decreased insect contact with the trap. Adult *O. surinamensis* are photonegative (Arbogast and Carthon 1973), and ambient light surrounding the arenas would cause the insects to move in toward the center of the arena, the location of the grain probe. This effect would be higher in the smaller arenas and thus may also contribute to an inverse relationship between insect capture and arena size.

Circadian rhythm has been documented in the foraging activity in *O. surinamensis* (Bell and Kerslake 1986). For insects held in a photoperiod of 15:9 (L:D) h, peaks in feeding activity occurred 2–6 h after the start of scotophase. They found that the cyclic periodicity continued when the insects were moved to continuous dark although the intervals were slightly <24 h. When the insects were moved to continuous light, activity cycles again continued, but the intervals were slightly longer than 24 h and activity peaks became more broad and the peaks were "substantially damped." Onset of scotophase was found to be the principle cue for entrainment of the foraging circadian rhythm. Role of the rearing photoperiod as the entrainment cue was also observed on the first day of our study. Although there was a 24-h lag before the initiation of the trial, during which the insects in the arenas were exposed to the ambient photoperiod, increase in capture frequency corresponded more closely with the scotophase from the rearing photoperiod than with the ambient scotophase in the two smaller arenas. Intervals between the peaks lengthened over the next 2 d and, by the last day, capture frequency corresponded more closely with the ambient photoperiod in the two larger arenas.

The EGPIC grain probe traps were designed to provide electronic counts that would indicate insect activity without the necessity of entering the grain storage unit. Models have been developed for estimating insect density in stored grain from trap count data (Lippert and Hagstrum 1987; Hagstrum et al. 1997, 1998), and additional information on factors such as environmental conditions are needed for input into expert systems such as the stored grain advisor (Flinn and Muir 1995). New EGPIC systems are being developed that facilitate species identification based on size of the electronic pulse generated by the falling insect (Shuman et al. 2003). Information on time period of capture, as was observed in our tests of *O. surinamensis,* obtained from the time-stamp data in EGPIC will add to the species identification capability of the new system. Availability of an electronic grain probe as part of an EGPIC system, which is combined with automated temperature and/or moisture monitoring systems, will provide additional information that can be used to improve trap interpretation and better management decisions.

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